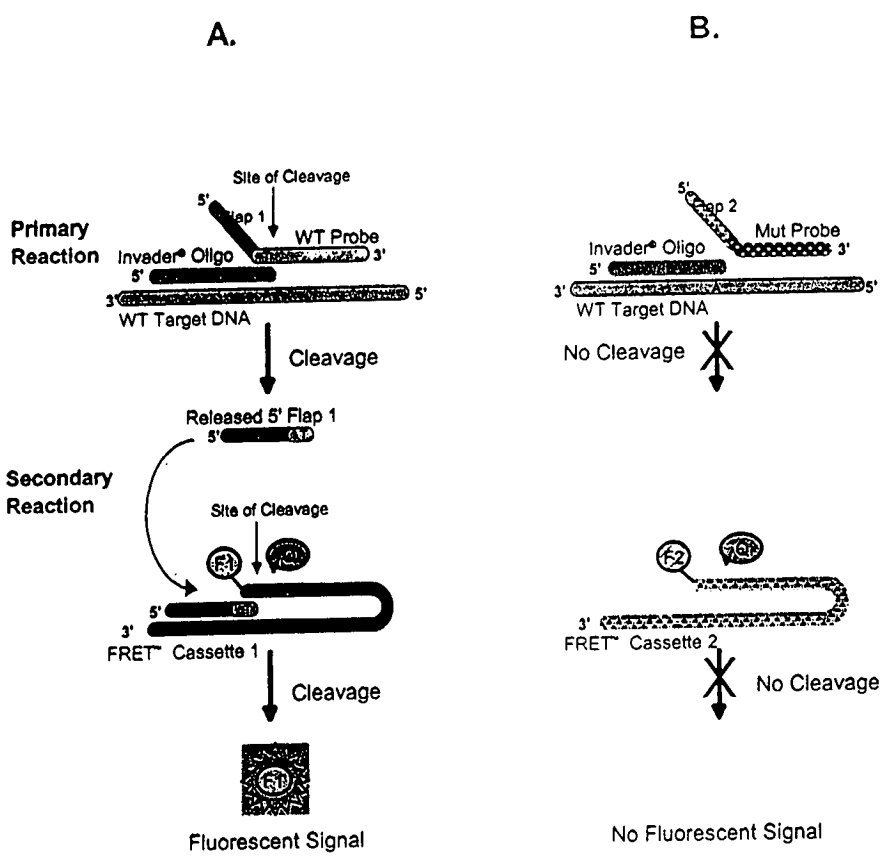


Fig. 1

Figure 2

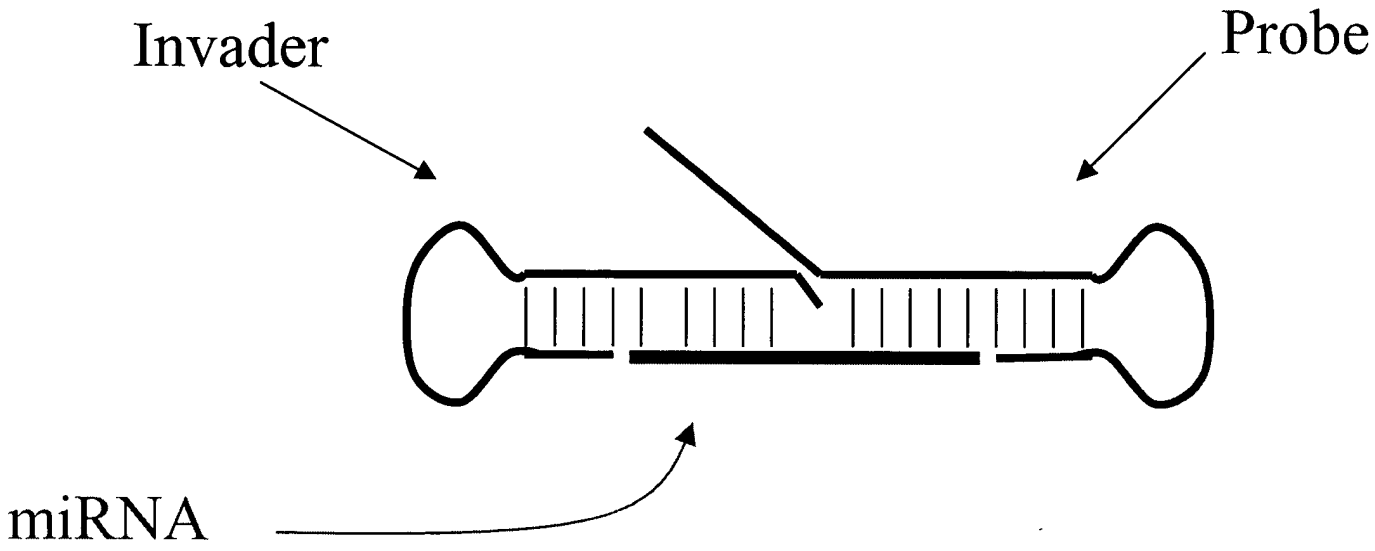


Figure 3

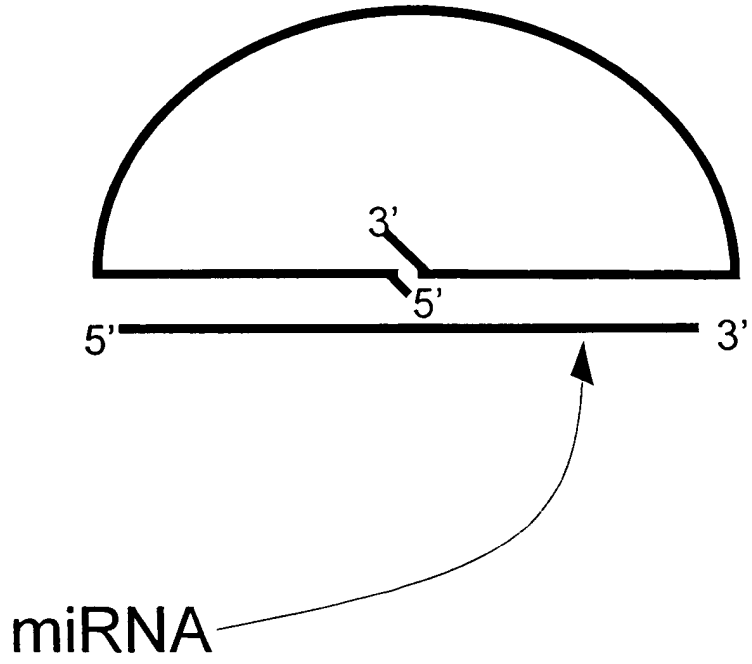
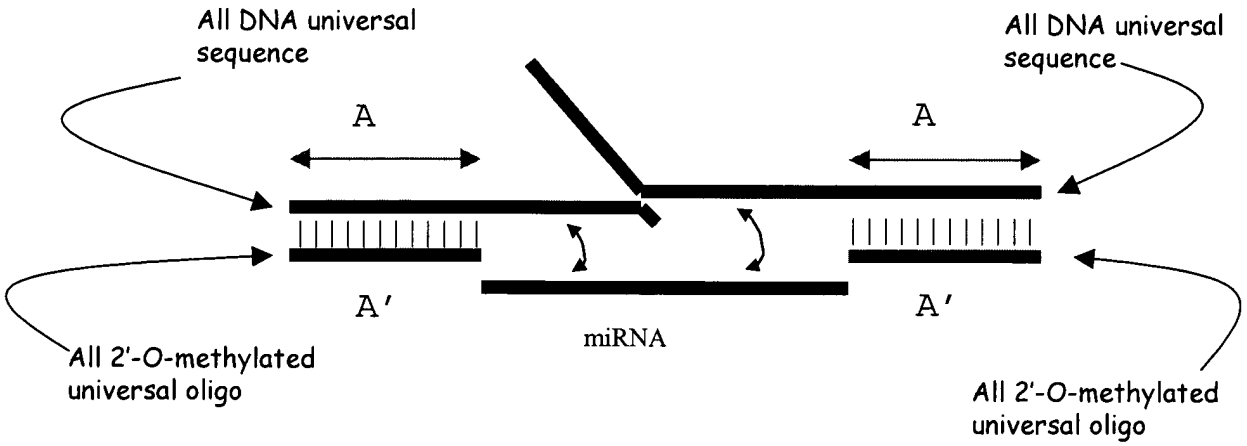


Figure 4



A = Universal sequence that is added to the 3' and 5' of probes and INVADER oligonucleotides, respectively.
From 5' to 3', the probe is composed of the 5'-flap, the miRNA complementary region, and the DNA universal sequence "A".
The INVADER oligonucleotide from 5' to 3', is composed of the DNA universal sequence "A" and an miRNA complementary region.

A' = 2'-O-methyl universal oligonucleotide that compliments the sequence "A" and is added to kits as a standard oligonucleotide.

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FIG. 5

SEQ ID NO	Target	Oligo type	Sequence (5'-3')
SEQ ID NO:1	human let-7 miRNA	Invader oligo	ggcacuuuugugccAACTATACAACCG
SEQ ID NO:2	human let-7 miRNA	probe oligo	CCGTCGCTGCGTTACTACCTCAcgacguuuucgucg
SEQ ID NO:3	human let-7 miRNA	arrestor oligo	cgacgaaaacgucgugagguaguaacgcag
SEQ ID NO:4	human let-7 miRNA	miRNA	ugagguaguagguuguauaguu
SEQ ID NO:5	human let-7 miRNA	Invader oligo	ggcacuuuugugccAACTATACAACT
SEQ ID NO:6	human let-7 miRNA	probe oligo	CCGTCGCTGCGTCTACTACCTCAcgacguuuucgucg
SEQ ID NO:7	human let-7 miRNA	arrestor oligo	cgacgaaaacgucgugagguaguagacgcag
SEQ ID NO:8	human let-7 miRNA	Invader oligo	ggcacuuuugugccAACTATACAAT
SEQ ID NO:9	human let-7 miRNA	probe oligo	AACGAGGCGCACCCTACTACCTCAcgacguuuucgucg
SEQ ID NO:10	human let-7 miRNA	arrestor oligo	cgacgaaaacgucgugagguaguagggugcgc
SEQ ID NO:11	human miR-1	Invader oligo	ggcagcuuuugcugccCTCCATACTTCTC
SEQ ID NO:12	human miR-1	probe oligo	AACGAGGCGCACTTACATTCCAcgagccuuuuggcucg
SEQ ID NO:13	human miR-1	arrestor oligo	cgagccaaaaggcucguggaauguaagugcgc
SEQ ID NO:14	human miR-1	miRNA	uggaauguaaagaaguauggag
SEQ ID NO:15	human miR-1	Invader oligo	ggcagcuuuugcugccCTCCATACTTCC
SEQ ID NO:16	human miR-1	probe oligo	AACGAGGCGCACTTTACATTCCAcgagccuuuuggcucg
SEQ ID NO:17	human miR-1	arrestor oligo	cgagccaaaaggcucguggaauguaaagugcgc
SEQ ID NO:18	human miR-1	Invader oligo	ggcagcuuuugcugccCTCCATACTTT
SEQ ID NO:19	human miR-1	probe oligo	AACGAGGCGCACCTTTACATTCCAcgagccuuuuggcucg
SEQ ID NO:20	human miR-1	arrestor oligo	cgagccaaaaggcucguggaauguaaaggugcgc
SEQ ID NO:21	FAM FRET	FRET probe	Yca-cXt-gct-tcg-tgg
SEQ ID NO:22	SRT	Secondary Reaction template	CCA GGA AGC AAG TGA CGC AGC GAC ggu
SEQ ID NO:23	human let-7 miRNA	Invader oligo	ggcacuuuugugccaaCTATACAAT
SEQ ID NO:24	human let-7c miRNA	miRNA	uugguauguuggaugauggagu
SEQ ID NO:25	human let-7e miRNA	miRNA	ugguacguuggaugauggagu
SEQ ID NO:26	human let-7f miRNA	miRNA	uugauauguuagaugauggagu
SEQ ID NO:27	human miR-135	Invader oligo	ccgagcgaaagcucggTTCACATAGGAATC
SEQ ID NO:28	human miR-135	probe oligo	AACGAGGCGCACAAAAAGCCATAcgagccgaaaggcucg
SEQ ID NO:29	human miR-135	arrestor oligo	cgagccuuucggcucguauggcuuuuugugcgc
SEQ ID NO:30	human miR-135	Invader oligo	ccgagcgaaagcucggTTCACATAGGAAC
SEQ ID NO:31	human miR-135	probe oligo	AACGAGGCGCACTAAAAAGCCATAcgagccgaaaggcucg
SEQ ID NO:32	human miR-135	arrestor oligo	cgagccuuucggcucguauggcuuuuuagugcgc
SEQ ID NO:33	human miR-135	Invader oligo	ccgagcgaaagcucggTTCACATAGGAC
SEQ ID NO:34	human miR-135	probe oligo	AACGAGGCGCACATAAAAAGCCATAcgagccgaaaggcucg
SEQ ID NO:35	human miR-135	arrestor oligo	cgagccuuucggcucguauggcuuuuuaugugcgc
SEQ ID NO:36	human miR-135	Invader oligo	ccgagcgaaagcucggTTCACATAGGC
SEQ ID NO:37	human miR-135	probe oligo	AACGAGGCGCACAATAAAAAGCCATAcgagccgaaaggcucg
SEQ ID NO:38	human miR-135	arrestor oligo	cgagccuuucggcucguauggcuuuuuauugugcgc

FIG 5

FIG. 5

SEQ ID NO	Target	Oligo type	Sequence (5'-3')
SEQ ID NO:39	human miR-16	miRNA	uagcagcacgtaaauauuggcg
SEQ ID NO:40	SRT	Secondary Reaction template	CCAGGAAGCAAGTGGAGGCGTGACggu
SEQ ID NO:41	human GAPDH	Invader oligo	ggaaucauauuGGAACATGTAAACCATC
SEQ ID NO:42	human GAPDH	probe oligo	CCGCCGAGATCACGTAGTTGAGGTC-NH2
SEQ ID NO:43	human GAPDH	arrestor oligo	gaccucaacuacgugauc
SEQ ID NO:44	human miR-125b	miRNA	ucccugagacccuaacuuguga
SEQ ID NO:45	U6 RNA	Invader oligo	GGCCATGCTAATCTTCA
SEQ ID NO:46	U6 RNA	probe oligo	CCGCCGAGATCACTCTGTATCGTTC-NH2
SEQ ID NO:47	U6 RNA	arrestor oligo	gaacgauacagagugauc
SEQ ID NO:48	RED FRET		Yct-cXt-tct-cag-tgc-g
SEQ ID NO:49	SRT	Secondary Reaction template	CCAGCAAGCAAGTGGTGATCTCGGCggu
SEQ ID NO:50	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACTACCTCA-NH2
SEQ ID NO:51	human let-7a miRNA	Invader oligo	AACTATACAACT
SEQ ID NO:52	human let-7a miRNA	probe oligo	CCGTCGCTGCGTTACTACCTCA-NH2
SEQ ID NO:53	human let-7a miRNA	Invader oligo	AACTATACAACCG
SEQ ID NO:54	human let-7a miRNA	arrestor oligo	ugagguaguagacgcag
SEQ ID NO:55	human miR-15	probe oligo	AACGAGGCGCACATGTGCTGCTAcgagccuuuuggcucg
SEQ ID NO:56	human miR-15	Invader oligo	ggcagcuuuugcugccCACAAACCATTC
SEQ ID NO:57	human miR-15	arrestor oligo	cgagccaaaaggcucguagcagcacaugugcgc
SEQ ID NO:58	human miR-15	probe oligo	AACGAGGCGCACATGTGCTGCTAGCTCGCCACGCCG-NH2
SEQ ID NO:59	human miR-15	Invader oligo	GCTCGCCACGCCGCACAAACCATTC
SEQ ID NO:60	human miR-15	stacker oligo	cggcguggcgagc
SEQ ID NO:61	human miR-15	arrestor oligo	cggcguggcgagcuagcagcacaugugcgc
SEQ ID NO:62	human miR-15	miRNA	uagcagcacauaaugguuugug
SEQ ID NO:63	human miR-135	probe oligo	AACGAGGCGCACAATAAAAAGCCATAGCTCGCCACGCCG-NH2
SEQ ID NO:64	human miR-135	Invader oligo	GCTCGCCACGCCGTTCACATAGGC
SEQ ID NO:65	human miR-135	arrestor oligo	cggcguggcgagcuauggcuuuuuauugugcgc
SEQ ID NO:66	human miR-15	arrestor oligo	uagcagcacaugugcgc
SEQ ID NO:67	human miR-15	probe oligo	AACGAGGCGCACATGTGCTGCTAGGCGAAGCC
SEQ ID NO:68	human miR-15	Invader oligo	GGCGAAGCCCACAAACCATTC
SEQ ID NO:69	human miR-15	probe oligo	AACGAGGCGCACATGTGCTGCTAGGCGAAgcc
SEQ ID NO:70	human miR-15	Invader oligo	ggcGAAGCCCACAAACCATTC
SEQ ID NO:71	human miR-15	probe oligo	AACGAGGCGCACATGTGCTGCTAggcuucggcc
SEQ ID NO:72	human miR-15	Invader oligo	ggcuucggccCACAAACCATTC
SEQ ID NO:73	human let-7a miRNA	Invader oligo	GGCACTTTTGTGCCAACTATACAACT
SEQ ID NO:74	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACTACCTCACGACGTTTTCGTCG
SEQ ID NO:75	human let-7a miRNA	Invader oligo	ggcacTTTTGTGCCAACTATACAACT
SEQ ID NO:76	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACTACCTCACGACGTTTTcgucg

FIG 5

FIG. 5

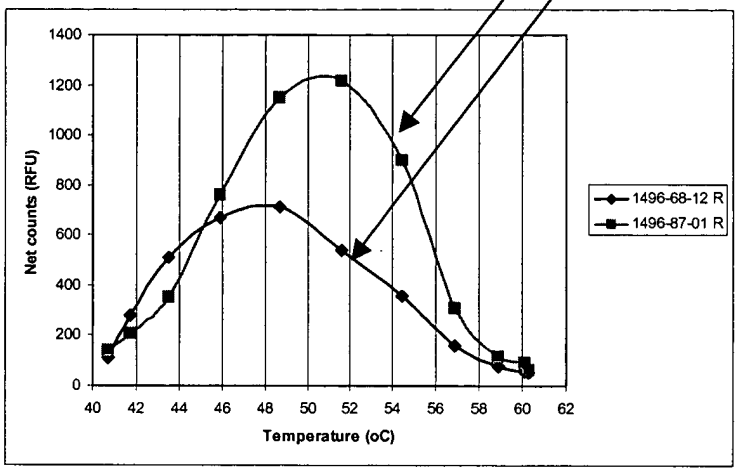
SEQ ID NO	Target	Oligo type	Sequence (5'-3')
SEQ ID NO:77	human miR-16 miRNA	Invader oligo	ggcagcuuuugcugccCGCCAATATTG
SEQ ID NO:78	human miR-16 miRNA	probe oligo	AACGAGGCGCACTACGTGCTGCTAcgagccuuuuggcucg
SEQ ID NO:79	human miR-16 miRNA	arrestor oligo	cgagccaaaaggcucguagcagcacguagugcgc
SEQ ID NO:80	human miR-125b miRNA	Invader oligo	ggcagcuuuugctgccTCACAAGTTAGA
SEQ ID NO:81	human miR-125b miRNA	probe oligo	AACGAGGCGCACGGTCTCAGGGAcgagccuuuuggcucg
SEQ ID NO:82	human miR-125b miRNA	arrestor oligo	cgagccaaaaggcucgucccugagaccgugcgc
SEQ ID NO:83	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACTACCTCAcgacguuuucgucgu
SEQ ID NO:84	human let-7a miRNA	Invader oligo	uggcacuuuugugccAACTATACAACT
SEQ ID NO:85	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACTACCTCAcgacguuuucguc
SEQ ID NO:86	human let-7a miRNA	Invader oligo	gcacuuuugugccAACTATACAACT
SEQ ID NO:87	precursor human let-7a	miRNA	gggcuuuggggugagguaguagguuguauaguuuggaauauuaccaccggugaacuau gcaauuuucuaccuuuccugaaguccc
SEQ ID NO:88	miR-124a 21nt	miRNA	uaaggcacgcggugaaugcca
SEQ ID NO:89	miR-124a 22nt	miRNA	uuaaggcacgcggugaaugcca
SEQ ID NO:90	miR-124a miRNA	probe oligo	CCGTCGCTGCGTCGCGTGCCTTAcgagccuuuuggcucg
SEQ ID NO:91	miR-124a miRNA	arrestor oligo	uaaggcacgcgacgcag
SEQ ID NO:92	miR-124a miRNA	Invader oligo	ggcagcuuuugcugccTGGCATTCACA
SEQ ID NO:93	U6 RNA	probe oligo	CCGCCGAGATCACCTAATCTTCTCTGTAT-NH2
SEQ ID NO:94	U6 RNA	Invader oligo	CATCCTTGCGCAGGGGCCATGA
SEQ ID NO:95	U6 RNA	arrestor oligo	auacagagaagauuaggugauc
SEQ ID NO:96	human miR-135	miRNA	uauggcuuuuuauuccuaugugaa
SEQ ID NO:97	human miR-1d	miRNA	uggaauguaaagaaguauguau
SEQ ID NO:98	human miR-1d	probe oligo	AACGAGGCGCACTTTACATTCCAcgagccuuuuggcucg
SEQ ID NO:99	human miR-1d	Invader oligo	ggcagcuuuugcugccATACATACTTCC
SEQ ID NO:100	human miR-1d	arrestor oligo	cgagccaaaaggcucguggaauguaaagugcgc
SEQ ID NO:101	human beta actin siRNA	probe oligo-antisense	AACGAGGCGCACAAGATCATTGCggcuucggcc
SEQ ID NO:102	human beta actin siRNA	Invader oligo-antisense	ggcuucggccAATGAAGATCC
SEQ ID NO:103	human beta actin siRNA	arrestor oligo-antisense	gcaaugaucuugugcgc
SEQ ID NO:104	human beta actin siRNA	probe oligo-sense	AACGAGGCGCACCTTGATCTTCAggcuucggcc
SEQ ID NO:105	human beta actin siRNA	Invader oligo-sense	ggcuucggccAAGCAATGATA
SEQ ID NO:106	human beta actin siRNA	arrestor oligo-sense	ugaagaucaaggugcgc

Figure 6
Design Optimization

flap forms single base invasion

flap forms two base invasion

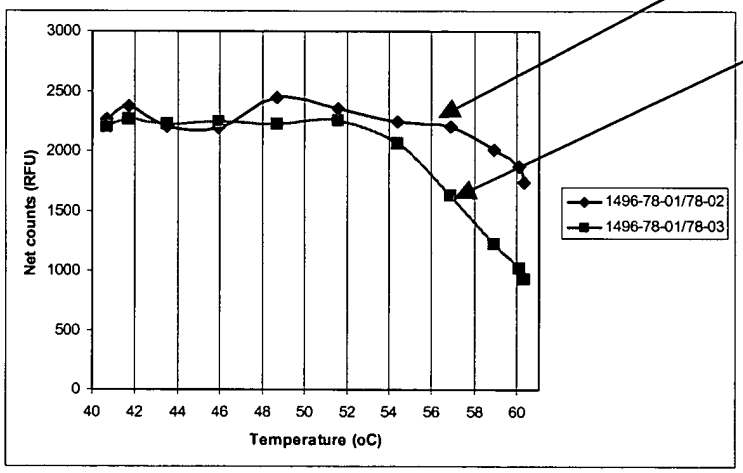
1496-87-01R



2'-O-methyl 5'-end of invader

Natural 5'-end of invader

1496-78-01 R



2'-O-methyl only at stacking region of invader

2'-O-methylation of invader extends into the miRNA base pairing region

1496-96-01 R

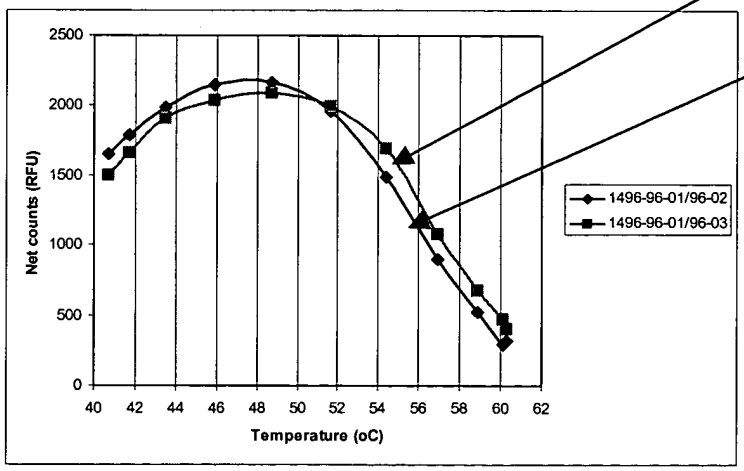


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Figure 7
Design Optimization

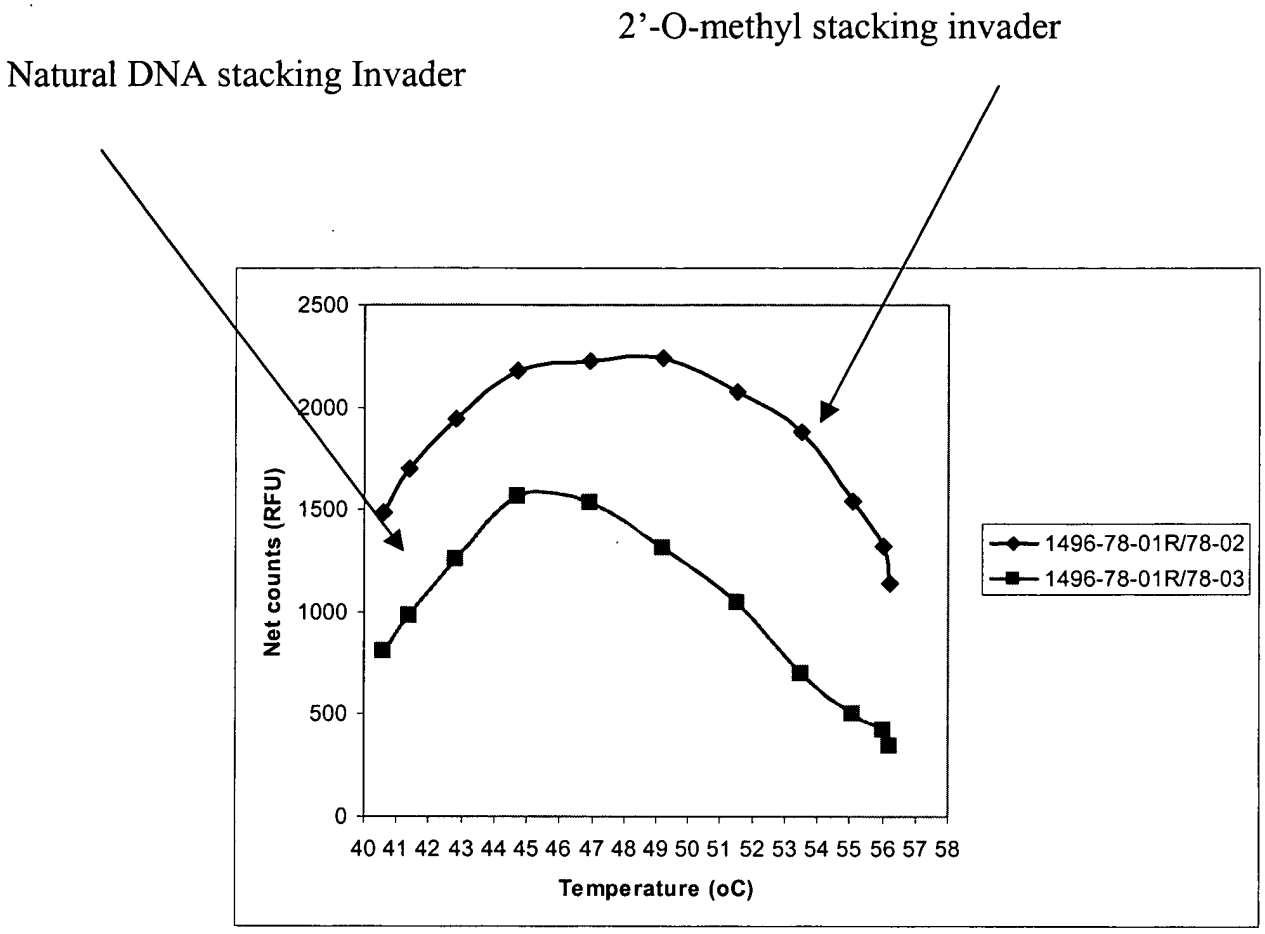

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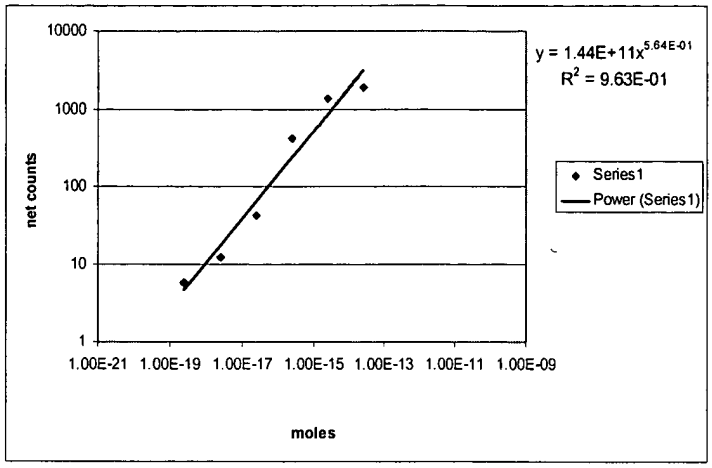
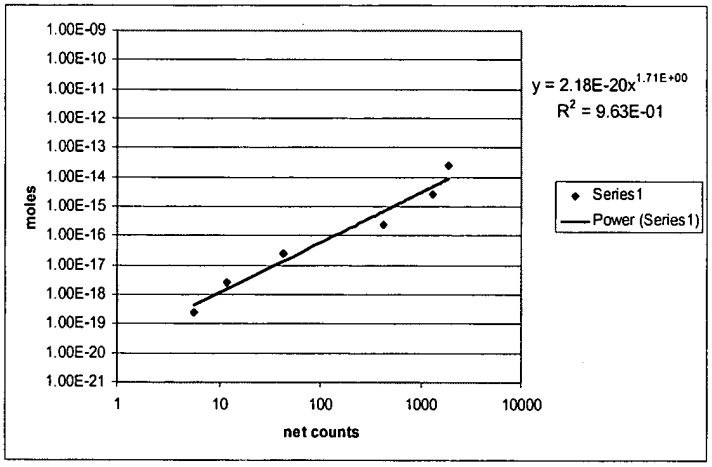
Figure 8
LOD let-7 (1496-78-01R)





a

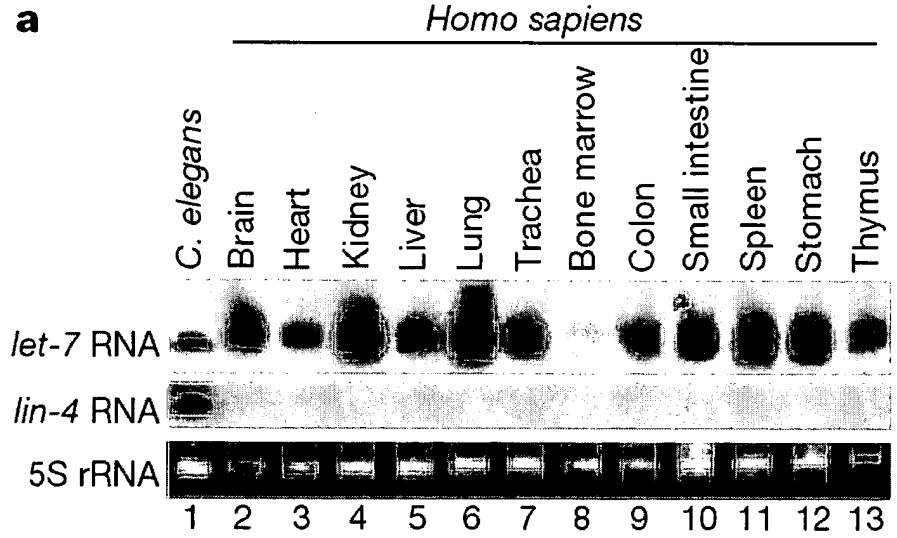


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Figure 9
cross reactivity let-7

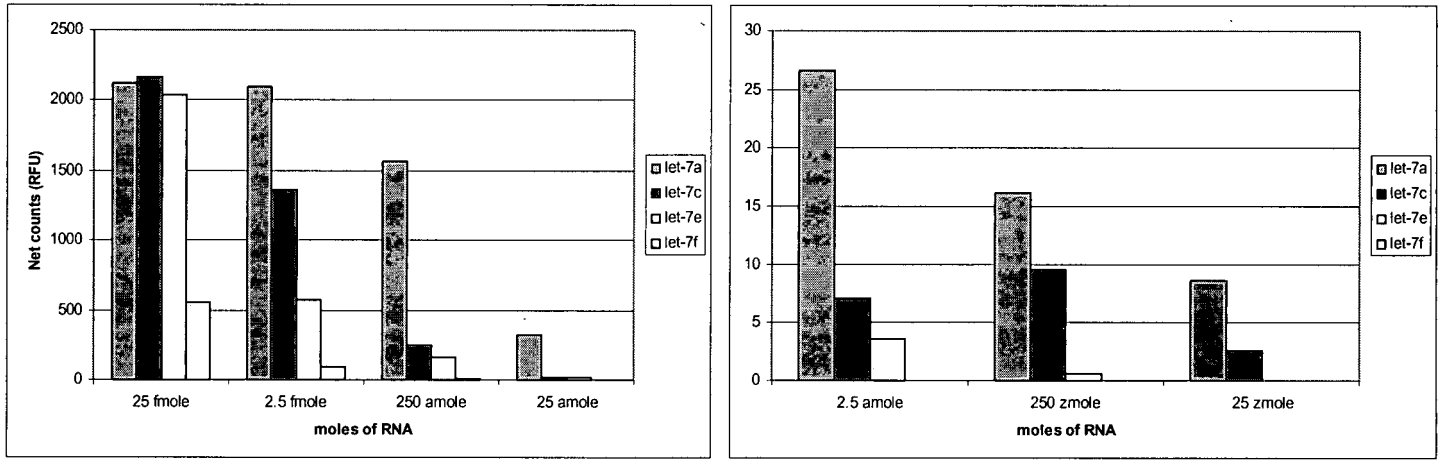

Figure 10
LOD mir-1

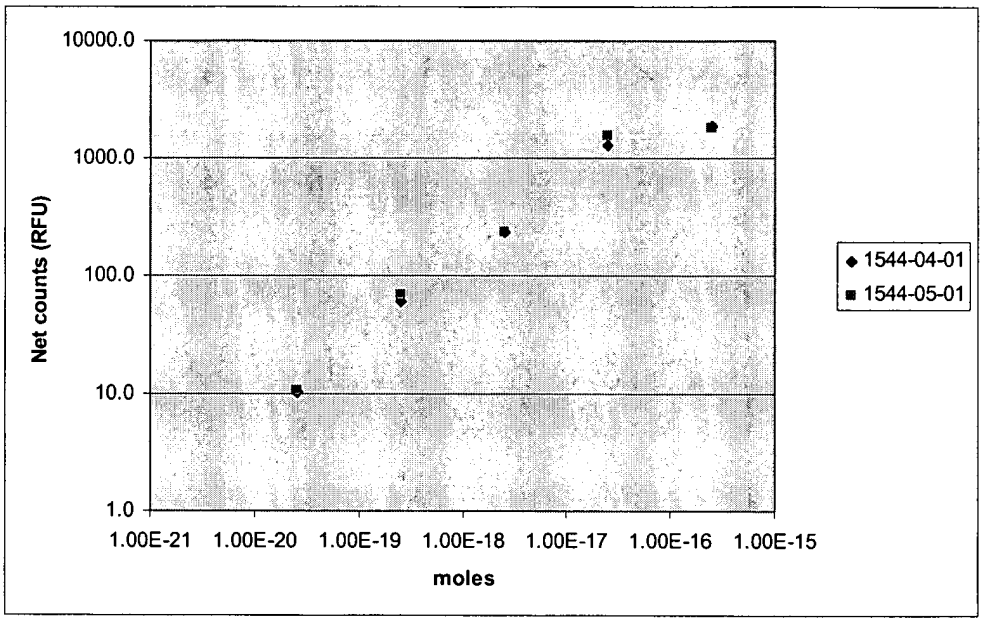

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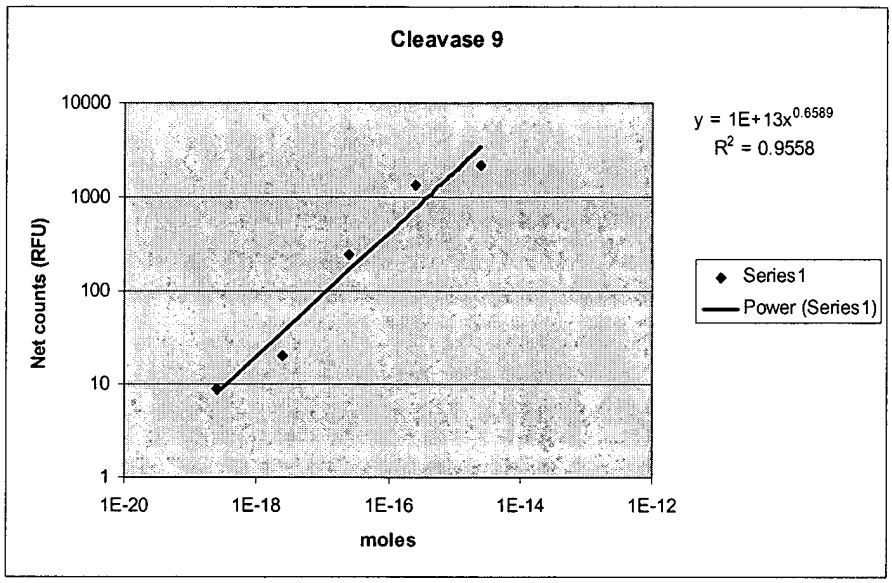
Figure 11
LOD let-7 (1496-78-01R) using CLEAVASE XII enzyme



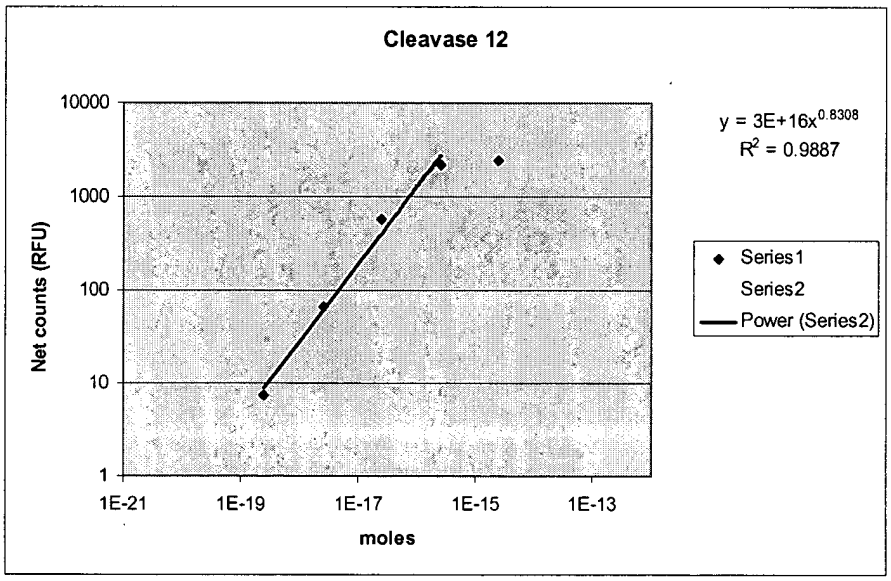


FIGURE 12

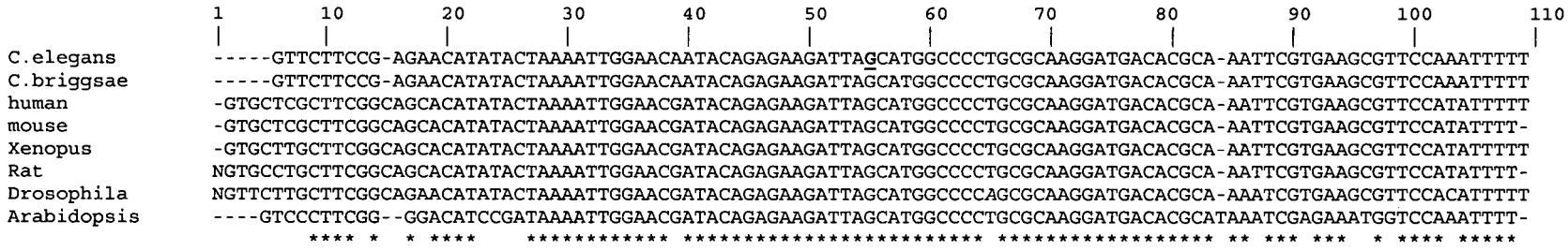


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Figure 13

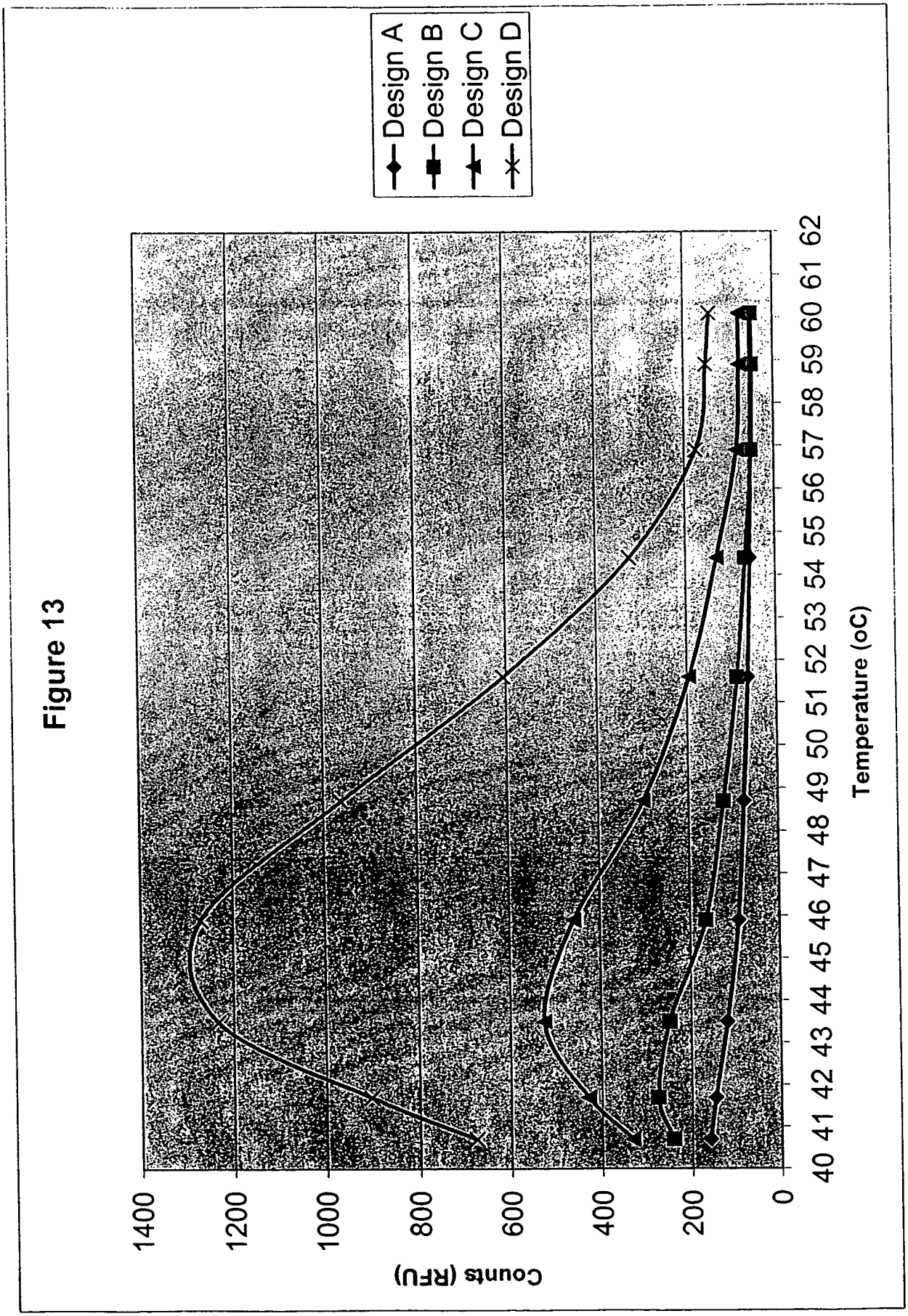


Figure 14

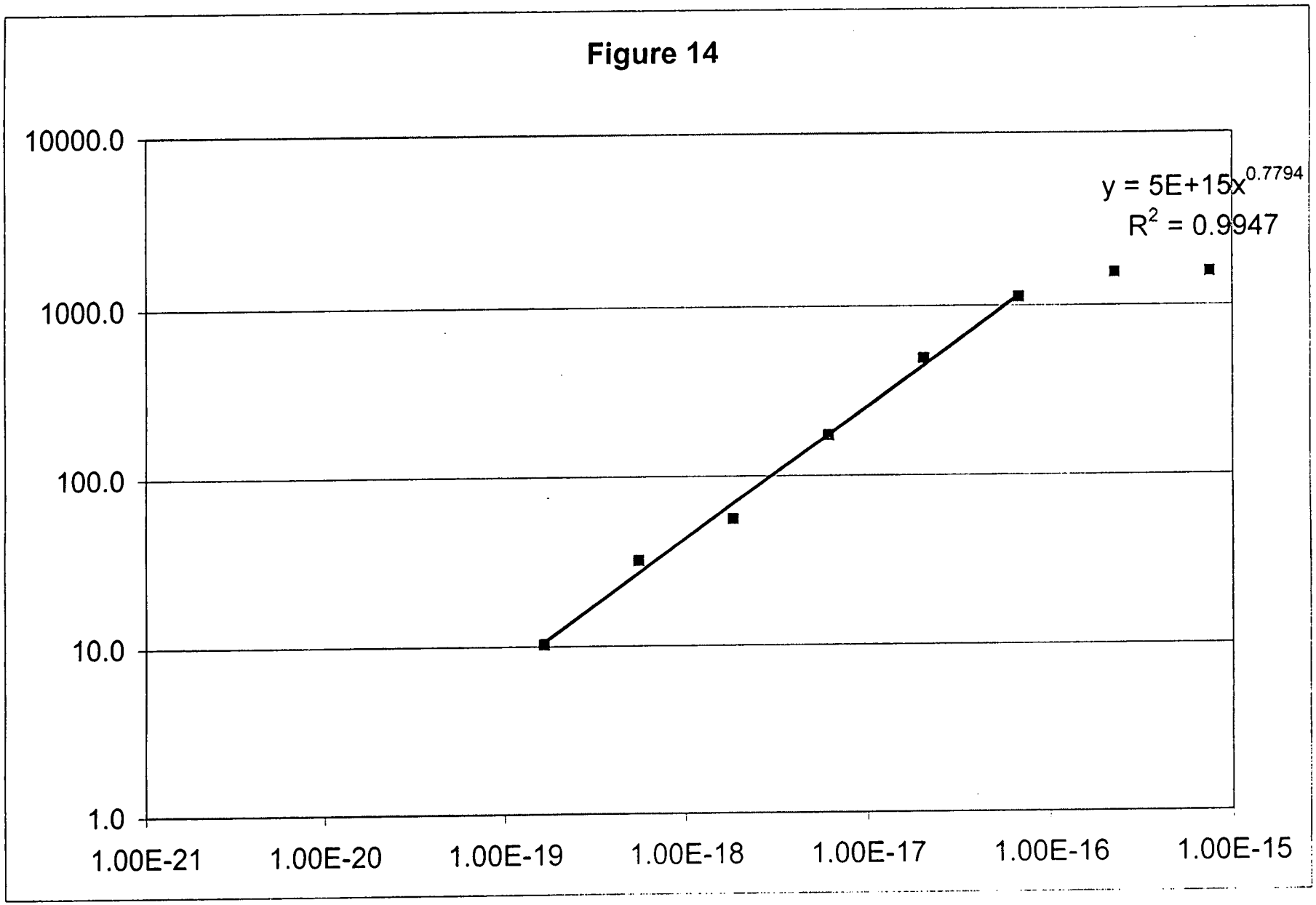

10000.0
1000.0
100.0
10.0
1.0
1.00E-21
1.00E-20
1.00E-19
1.00E-18
1.00E-17
1.00E-16
1.00E-15
y = 5E+15x^0.7794
R^2 = 0.9947

Fig. 15

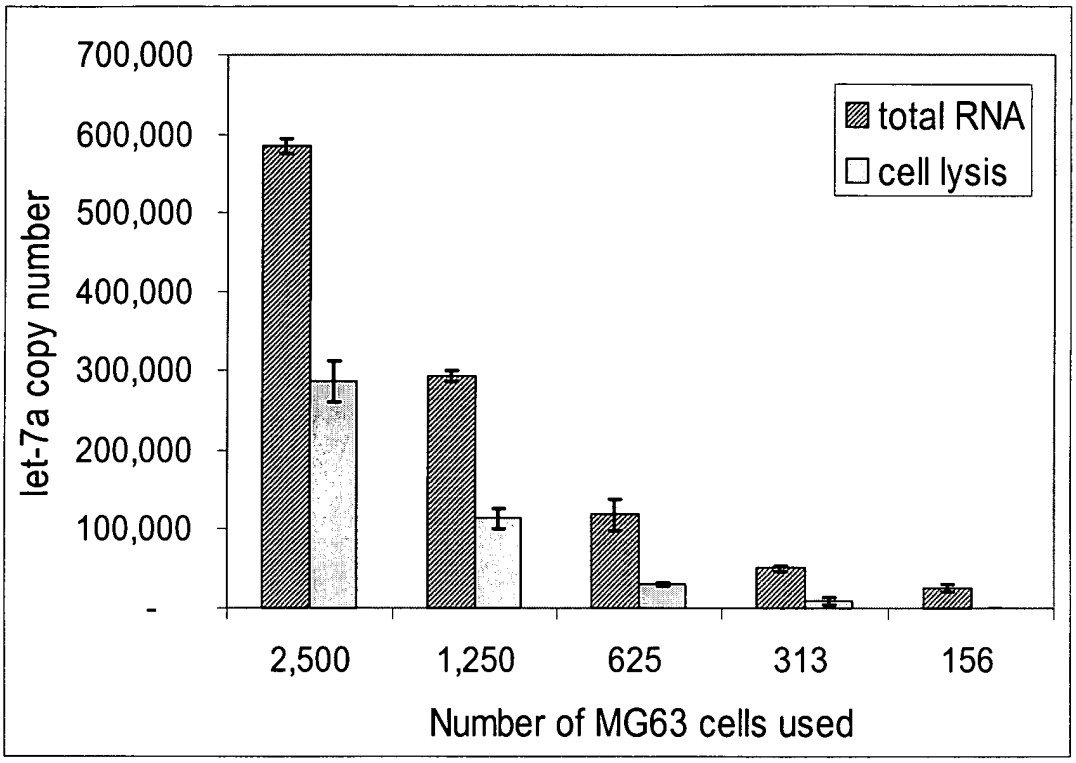

700,000
600,000
500,000
400,000
300,000
200,000
100,000
-
let-7a copy number
total RNA
cell lysis
2,500
1,250
625
313
156
Number of MG63 cells used

Figure 16

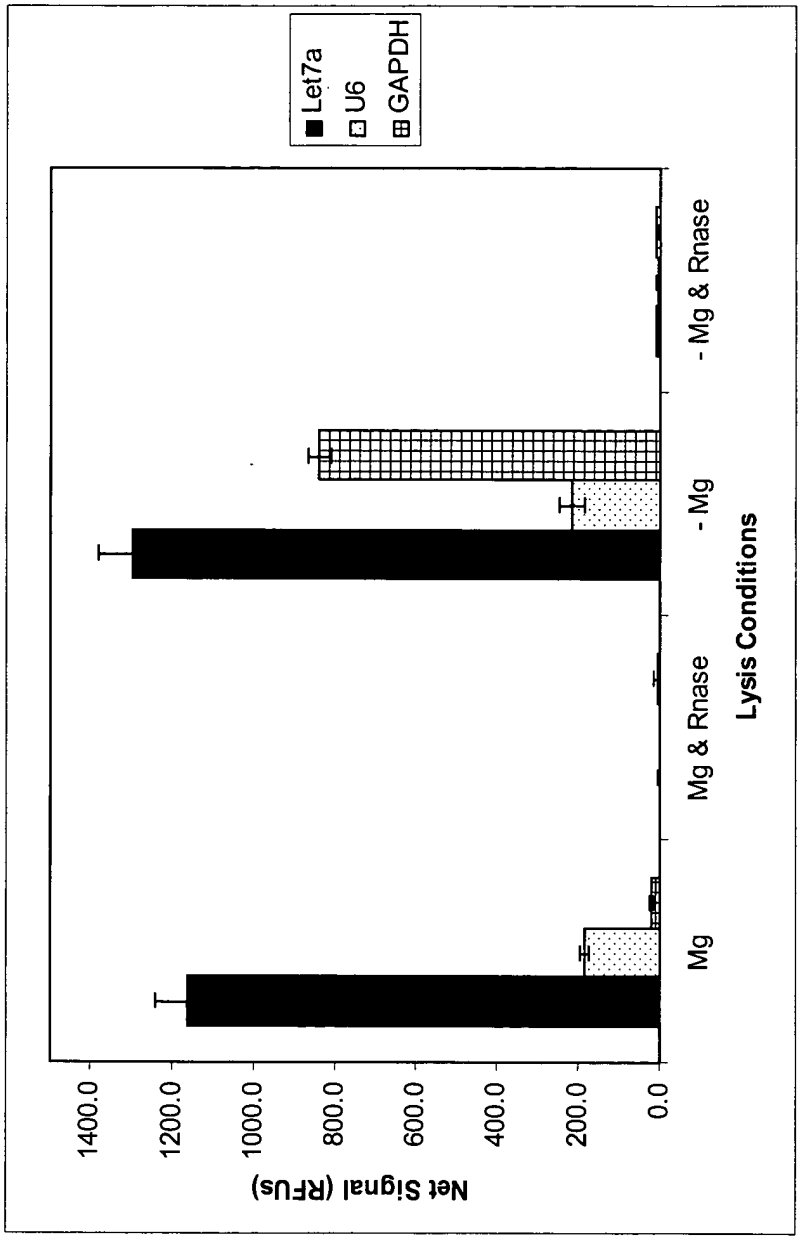

Let7a
U6
GAPDH
Net Signal (RFUs)
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Mg
Mg & Rnase
- Mg
- Mg & Rnase
Lysis Conditions

Figure 17

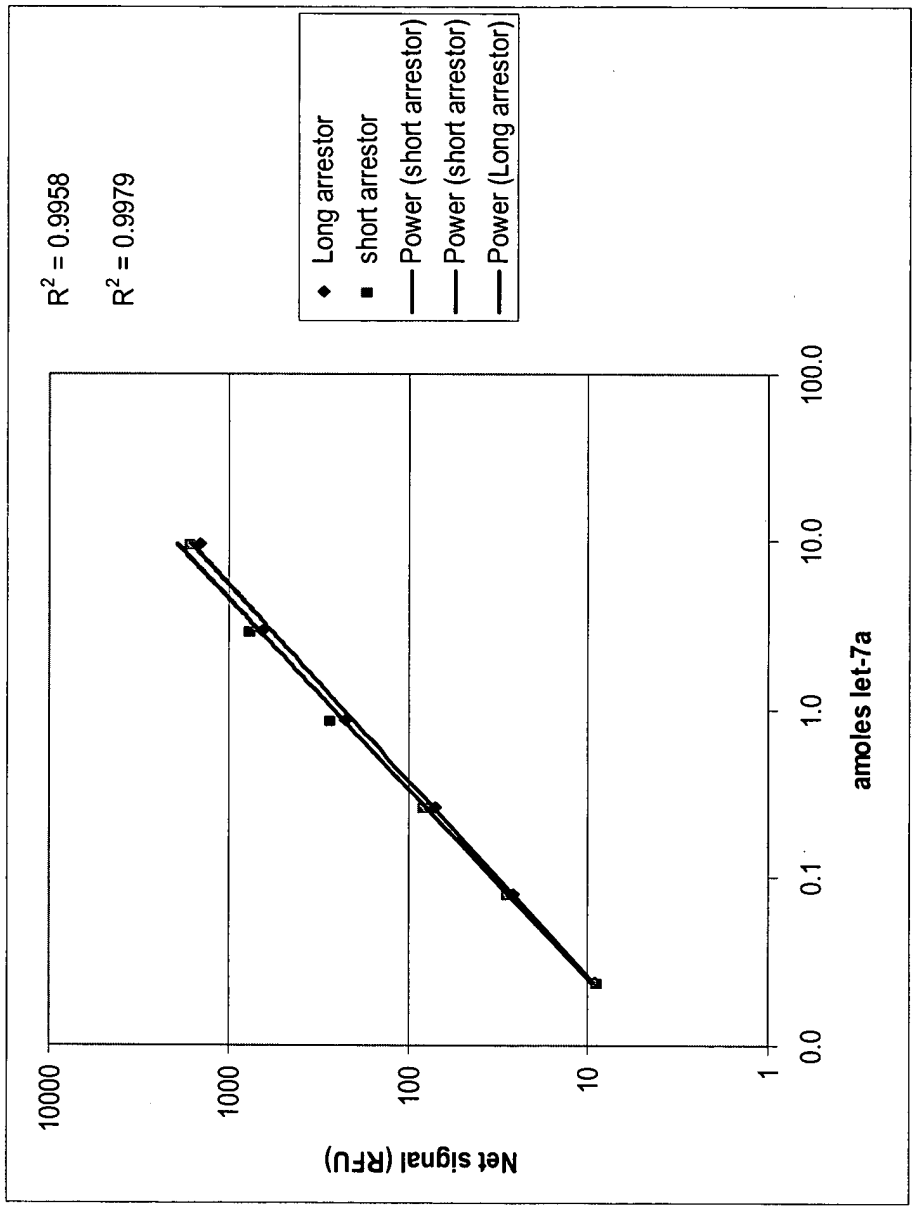
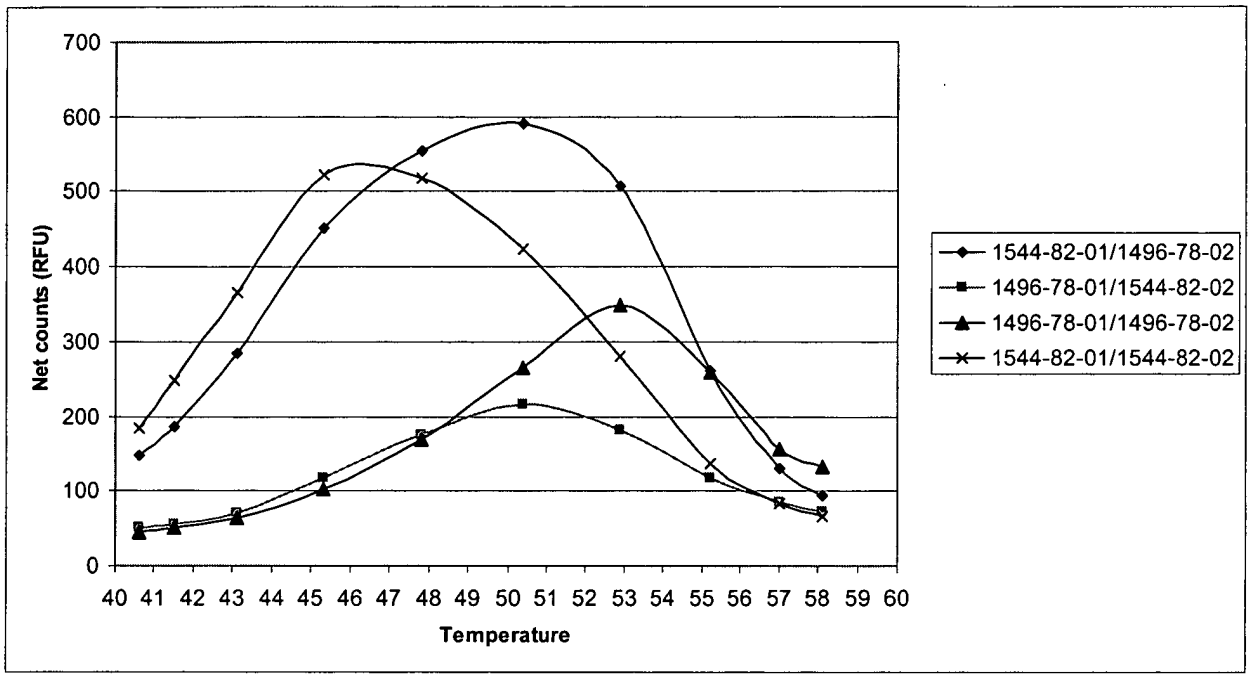
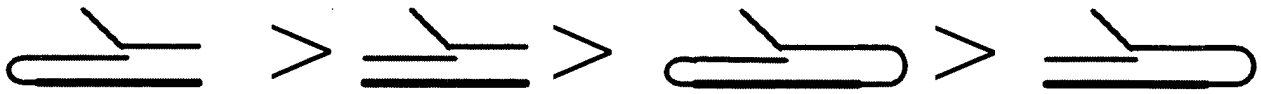

R^2 = 0.9958
R^2 = 0.9979
Long arrestor
short arrestor
Power (short arrestor)
Power (short arrestor)
Power (Long arrestor)
Net signal (RFU)
10000
1000
100
10
1
0.0
0.1
1.0
10.0
100.0
amoles let-7a

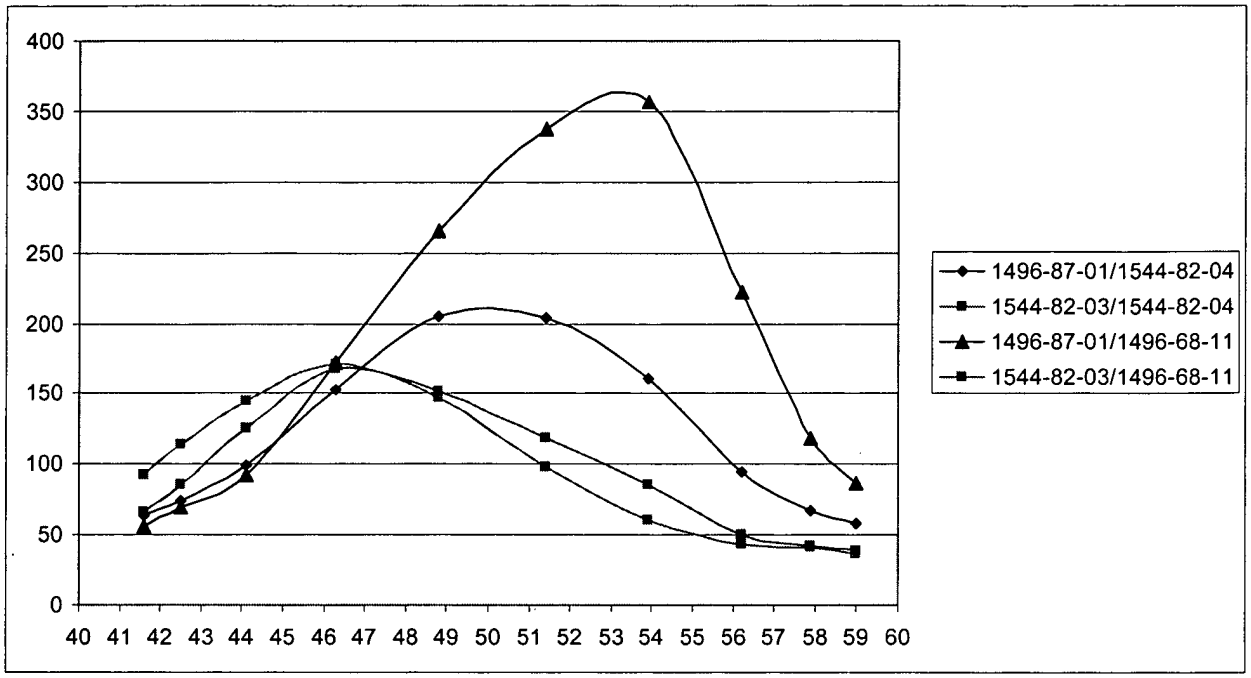
FIGURE 18

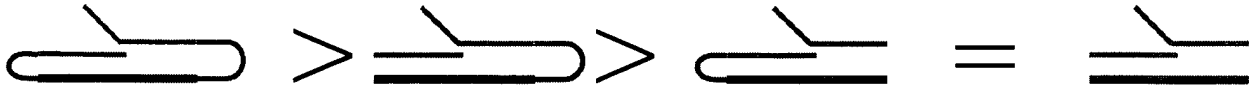




Normalized maximum performance	100%	90%	60%	30%
optimum temperature at maximum performance	50 °C	46 °C	53 °C	50 °C

Fig. 19





Normalized maximum performance →	100%	60%	50%	50%
optimum temperature at maximum performance →	53 °C	51 °C	47 °C	46 °C

Fig. 20

		let-7a moles	3.68E-17	1.10E-17	3.31E-18	9.94E-19	2.98E-19	8.95E-20	2.68E-20	NTC
Probe	*Invader*									
1496-78-01R	*1496-78-02*		1008	996	755	355	146	79	50	37
			991	1014	759	359	149	78	52	40
			1031	1056	774	355	156	79	50	40
			1054	1011	753	366	156	79	51	42
		average	**1021**	**1019**	**760**	**359**	**152**	**79**	**51**	**39.8**
		net	**981**	**980**	**721**	**319**	**112**	**39**	**11**	
		foz	**25.69**	**25.64**	**19.13**	**9.03**	**3.82**	**1.98**	**1.28**	
		stdev	**27.43**	**25.73**	**9.50**	**5.19**	**5.06**	**0.50**	**0.96**	
Probe	*Invader*									
1544-82-01	*1496-78-02*		1014	1079	933	575	244	103	52	31
			1054	1088	957	555	245	103	52	32
			1079	1074	971	592	250	113	53	33
			1079	1057	964	601	257	110	57	35
		average	**1057**	**1075**	**956**	**581**	**249**	**107**	**54**	**32.8**
		net	**1024**	**1042**	**924**	**548**	**216**	**75**	**21**	
		foz	**32.26**	**32.81**	**29.20**	**17.73**	**7.60**	**3.27**	**1.63**	
		stdev	**30.69**	**13.03**	**16.52**	**20.27**	**5.94**	**5.06**	**2.38**	

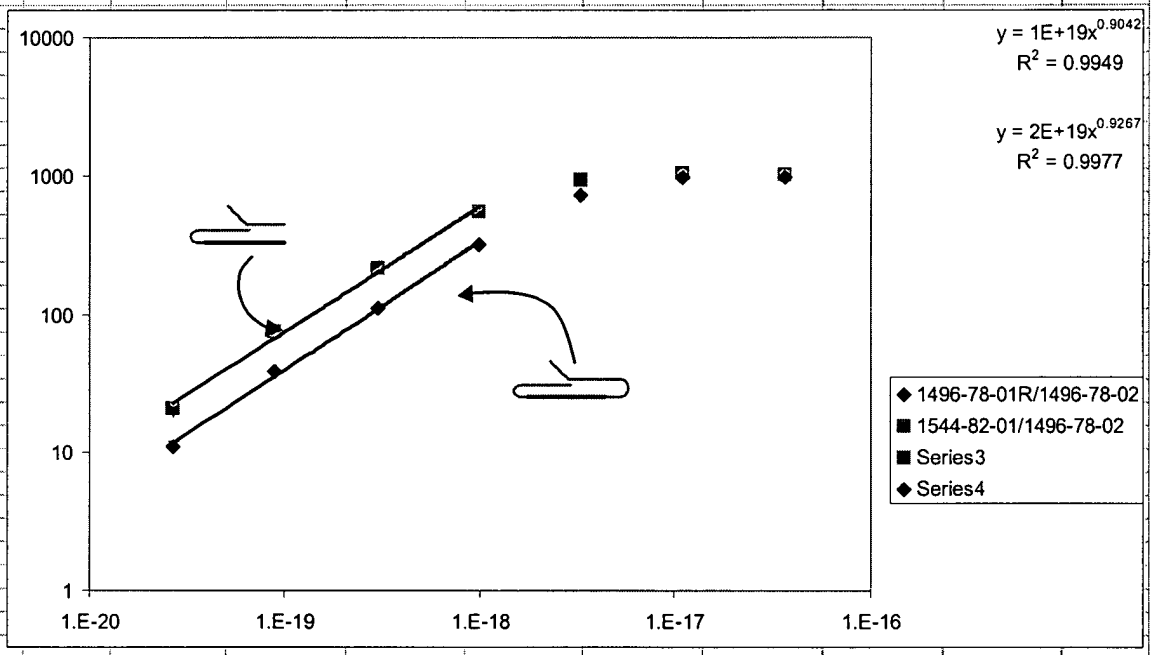


Figure 21

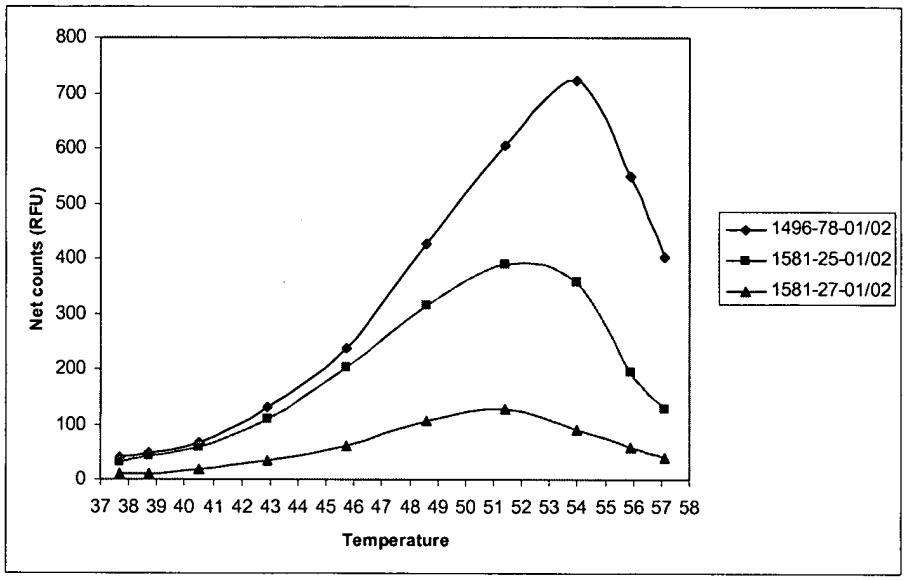
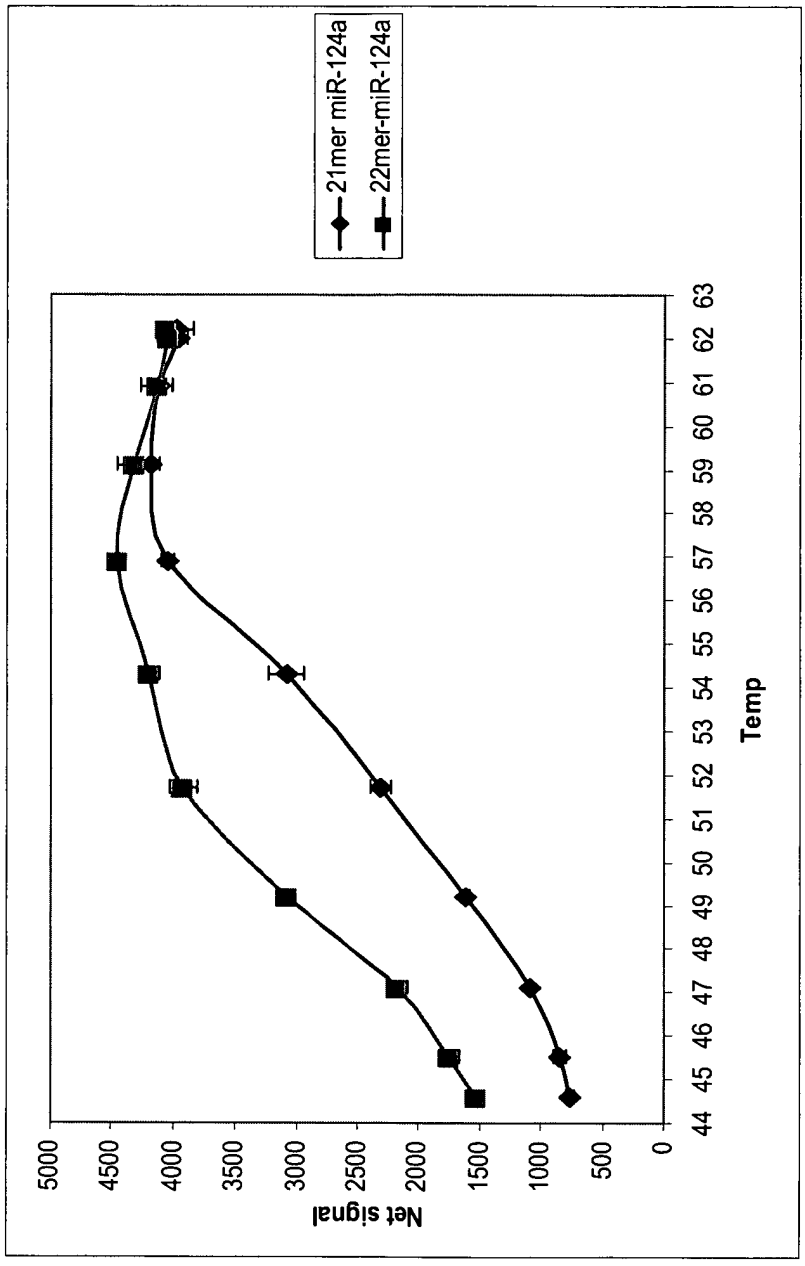

800
700
600
500
400
300
200
100
0
Net counts (RFU)
37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58
Temperature
1496-78-01/02
1581-25-01/02
1581-27-01/02

Figure 22


21mer miR-124a
22mer-miR-124a
Net signal
Temp
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63

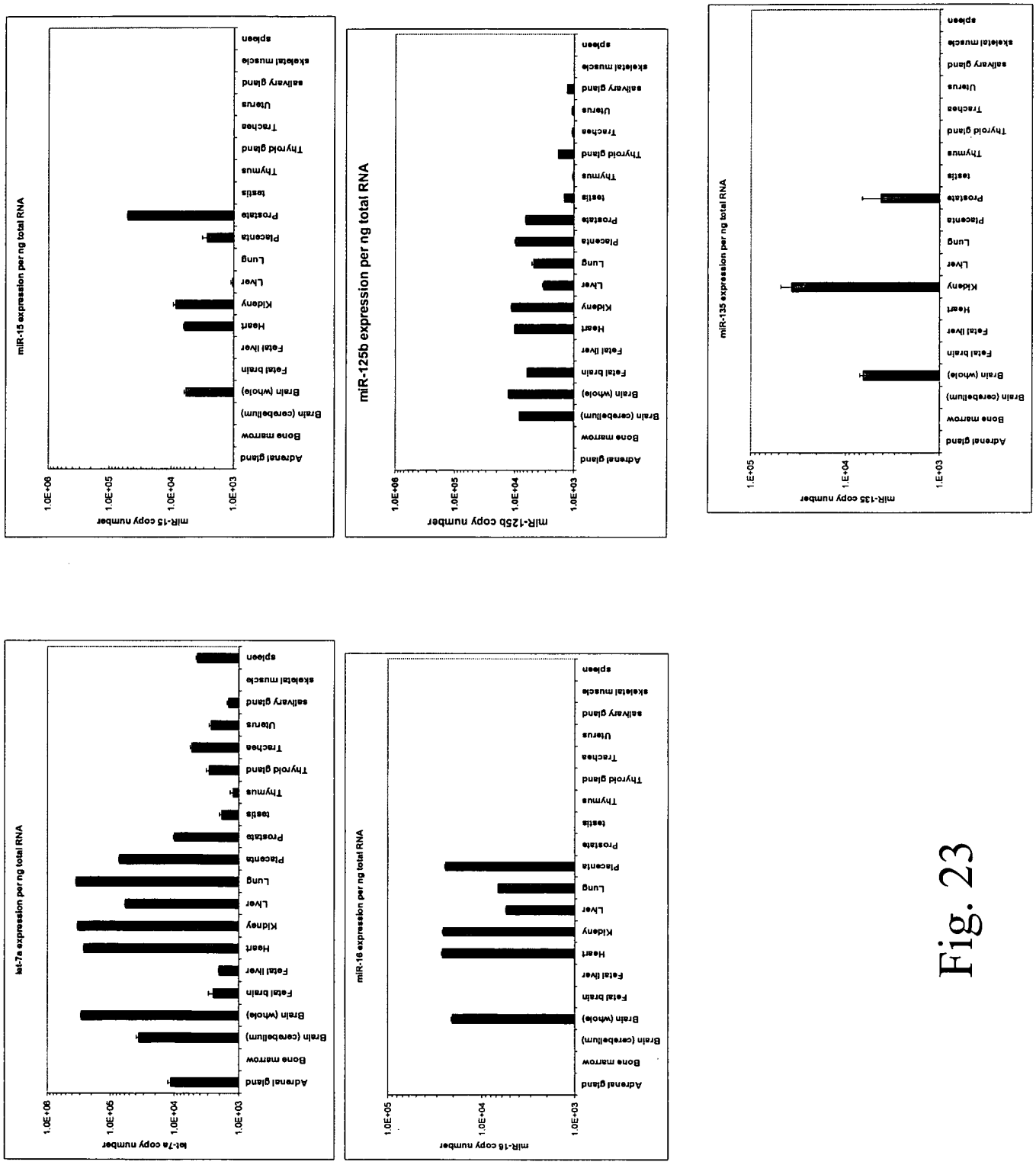
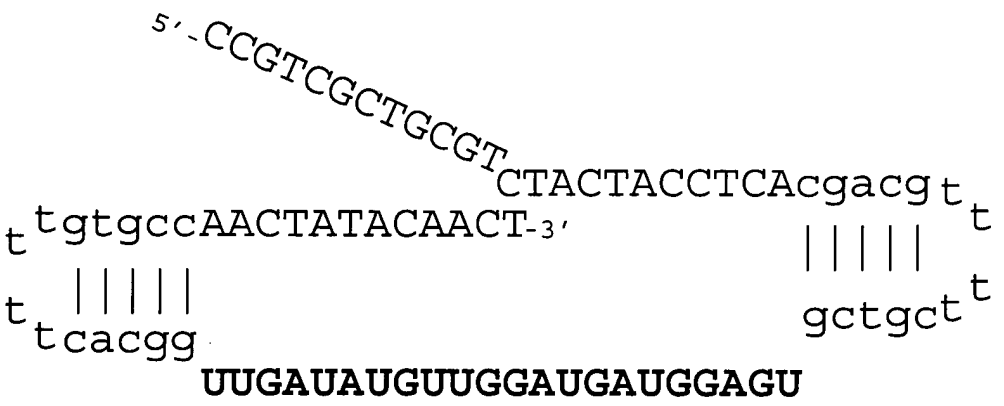
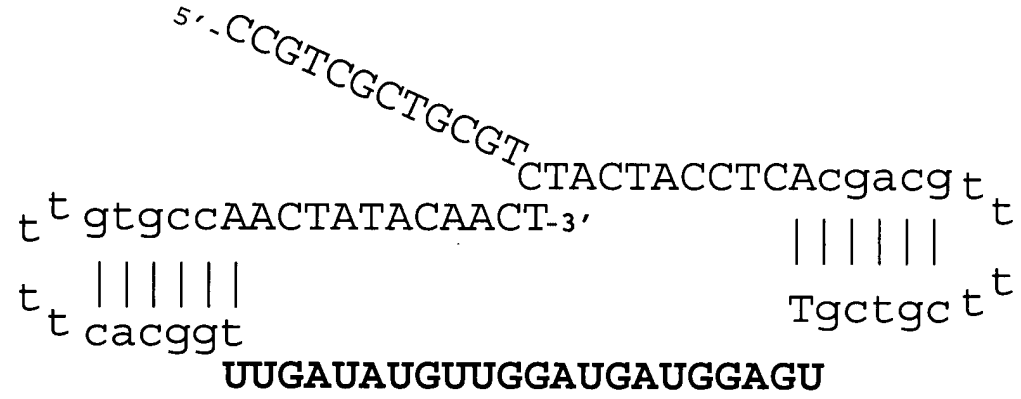

miR-15 expression per ng total RNA
miR-15 copy number
1.0E+06
1.0E+05
1.0E+04
1.0E+03
Adrenal gland
Bone marrow
Brain (cerebellum)
Brain (whole)
Fetal brain
Fetal liver
Heart
Kidney
Liver
Lung
Placenta
Prostate
testis
Thymus
Thyroid gland
Trachea
Uterus
salivary gland
skeletal muscle
spleen
miR-125b expression per ng total RNA
miR-125b copy number
1.0E+06
1.0E+05
1.0E+04
1.0E+03
Adrenal gland
Bone marrow
Brain (cerebellum)
Brain (whole)
Fetal brain
Fetal liver
Heart
Kidney
Liver
Lung
Placenta
Prostate
testis
Thymus
Thyroid gland
Trachea
Uterus
salivary gland
skeletal muscle
spleen
miR-135 expression per ng total RNA
miR-135 copy number
1.E+05
1.E+04
1.E+03
Adrenal gland
Bone marrow
Brain (cerebellum)
Brain (whole)
Fetal brain
Fetal liver
Heart
Kidney
Liver
Lung
Placenta
Prostate
testis
Thymus
Thyroid gland
Trachea
Uterus
salivary gland
skeletal muscle
spleen
let-7a expression per ng total RNA
let-7a copy number
1.0E+06
1.0E+05
1.0E+04
1.0E+03
Adrenal gland
Bone marrow
Brain (cerebellum)
Brain (whole)
Fetal brain
Fetal liver
Heart
Kidney
Liver
Lung
Placenta
Prostate
testis
Thymus
Thyroid gland
Trachea
Uterus
salivary gland
skeletal muscle
spleen
miR-16 expression per ng total RNA
miR-16 copy number
1.0E-05
1.0E-04
1.0E-03
Adrenal gland
Bone marrow
Brain (cerebellum)
Brain (whole)
Fetal brain
Fetal liver
Heart
Kidney
Liver
Lung
Placenta
Prostate
testis
Thymus
Thyroid gland
Trachea
Uterus
salivary gland
skeletal muscle
spleen

Fig. 23

Fig. 24


5'-CCGTCGCTGCGTCTACTACCTCAcgacg
ttgtgccAACTATACAACT-3'
ttcacgg
gctgc
UUGAUAUGUUGGAUGAUGGAGU


5'-CCGTCGCTGCGTCTACTACCTCAcgacg
ttgtgccAACTATACAACT-3'
ttcacggt
Tgctgc
UUGAUAUGUUGGAUGAUGGAGU

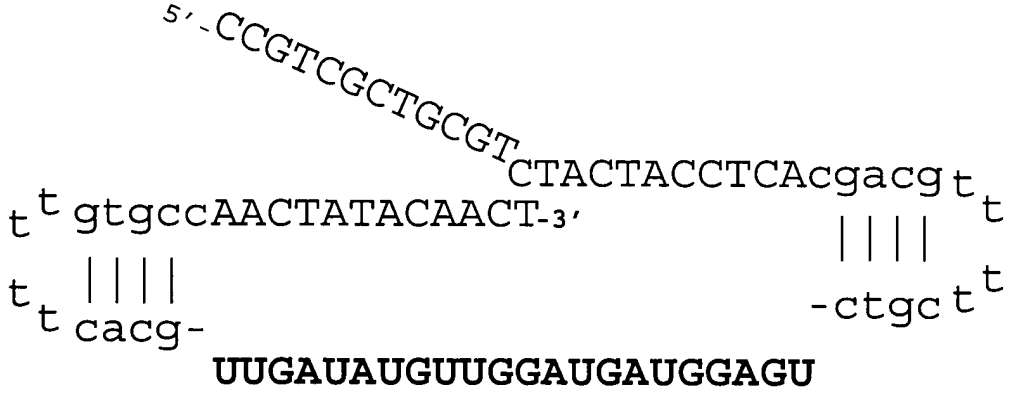
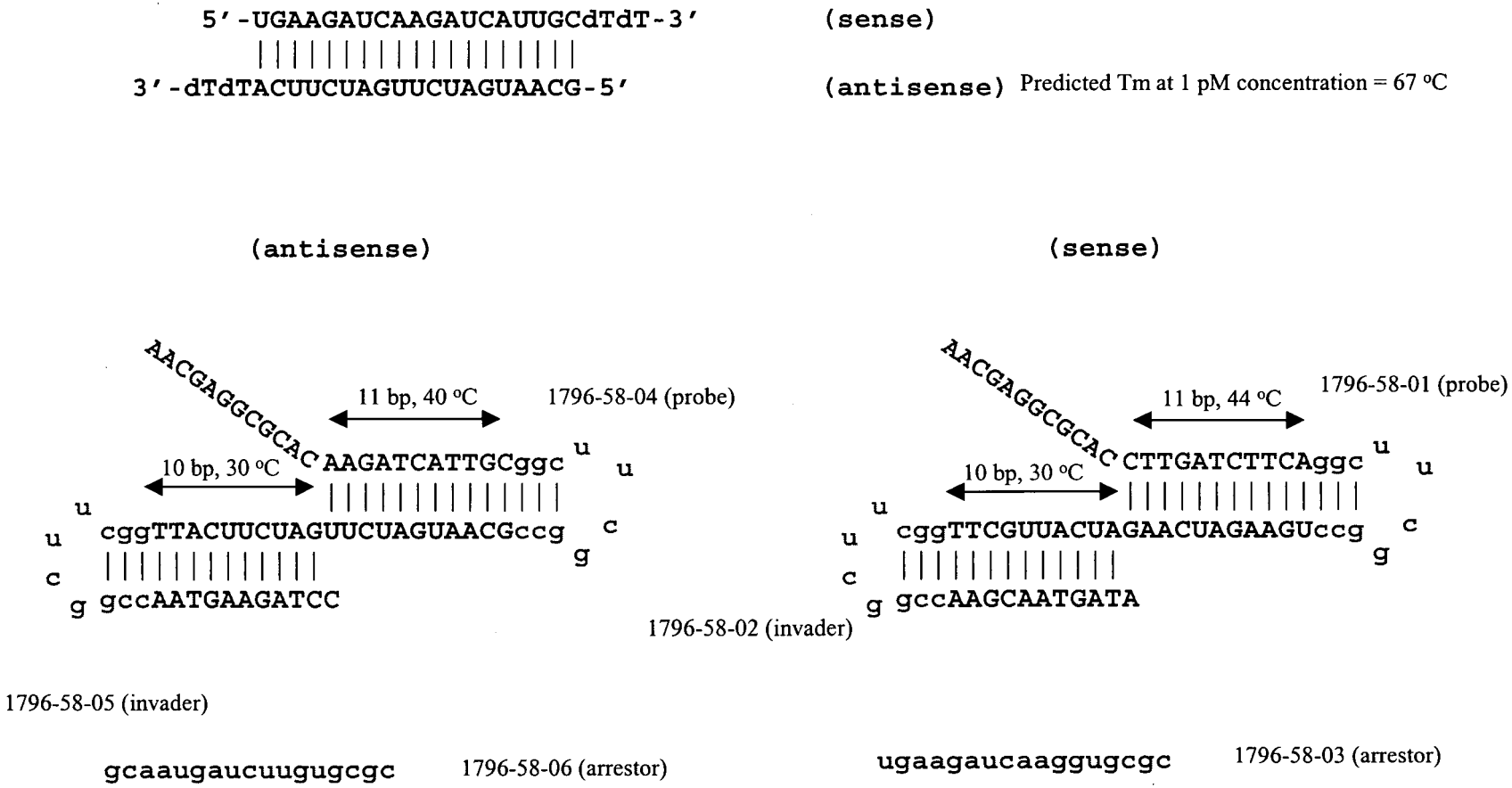

5'-CCGTCGCTGCGTCTACTACCTCAcgacg
ttgtgccAACTATACAACT-3'
ttcacg-
-ctgc
UUGAUAUGUUGGAUGAUGGAGU

Figure 25


5′-UGAAGAUCAAGAUCAUUGCdTdT-3′
(sense)
3′-dTdTACUUCUAGUUCUAGUAACG-5′
(antisense)
Predicted Tm at 1 pM concentration = 67 °C
(antisense)
(sense)
AACGAGGCGCAC
11 bp, 40 °C
1796-58-04 (probe)
10 bp, 30 °C
AAGATCATTGCggc
cggTTACUUCUAGUUCUAGUAACGccg
gccAATGAAGATCC
1796-58-02 (invader)
AACGAGGCGCAC
11 bp, 44 °C
1796-58-01 (probe)
10 bp, 30 °C
CTTGATCTTCAggc
cggTTCGUUACUAGAACUAGAAGUccg
gccAAGCAATGATA
1796-58-05 (invader)
gcaaugaucuugugcgc
1796-58-06 (arrestor)
ugaagaucaaggugcgc
1796-58-03 (arrestor)